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Glucose-induced MAPK signalling influences NeuroD1-mediated activation and nuclear localization

Helle V. Petersen[a,*], Jan N. Jensen[a,1], Roland Stein[b], Palle Serup[a]

[a] *Hagedorn Research Institute, Niels Steensensvej 6, DK-2820 Gentofte, Denmark*
[b] *Vanderbilt Medical Center, Department of Molecular Physiology and Biophysics, 723 Light Hall, Nashville, TN 37232, USA*

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Abstract **The helix–loop–helix transcription factor NeuroD1 (also known as Beta2) is involved in β-cell survival during development and insulin gene transcription in adults. Here we show NeuroD1 is primarily cytoplasmic at non-stimulating glucose concentrations (i.e. 3 mM) in MIN6 β-cells and nuclear under stimulating conditions (i.e. 20 mM). Quantification revealed that NeuroD1 was in 40–45% of the nuclei at 3 mM and 80–90% at 20 mM. Treatment with the MEK inhibitor PD98059 or substitution of a serine for an alanine at a potential mitogen-activated protein kinase phosphorylation site (S274) in NeuroD1 significantly increased the cytoplasmic level at 20 mM glucose. The rise in NeuroD1-mediated transcription in response to glucose also correlated with the change in sub-cellular localization, a response attenuated by PD98059. The data strongly suggest that glucose-stimulation of the MEK–ERK signalling pathway influences NeuroD1 activity at least partially through effects on sub-cellular localization.**

Key words: Insulin gene; Transcription; Phosphorylation; Regulation

1. Introduction

Insulin produced from pancreatic islet β-cells plays a vital role in the maintenance of glucose homeostasis. Insulin is uniquely expressed in β-cells where elevated glucose levels lead to increased insulin transcription, biosynthesis, and secretion. The *cis*-acting elements controlling insulin gene expression are located within the mammalian insulin enhancer region, which is found between nucleotides −340 and −91 relative to the transcription start site [1,2]. Several key control elements within this region have been identified, including the conserved C2 (−317 to −311 base pairs (bp) [3], A3 (−201 to −196 bp) [4,5], C1/RIPE3b (−118 to −107 bp) [6,7] and E1 (−100 to −91 bp) [8–11] elements. Mutations that decrease the binding affinity of the A3, C1/RIPE3b, and E1 activators reduce both glucose-regulated and β-cell-type-specific transcription from insulin gene reporter constructs [12–16].

The activator of insulin A3 element stimulated transcription is the PDX-1 homeodomain protein (also known as IPF-1, STF-1, and IDX-1 [17–19]). Endogenous insulin gene expression is activated upon ectopic expression of PDX-1 in α-cell lines [20,21], while profoundly reduced upon ablating pdx1 expression selectively in β-cells of mice in vivo [22]. Elevated glucose levels induce the PDX-1 binding and *trans*-activation domain (AD) activity [23–25]. PDX-1 is phosphorylated in response to glucose, although it is unclear if PI3 kinase [26,27] or p38 SAPK [28] is directly involved in these signalling events. Although the insulin C1 element activator, RIPE3b1, has not been isolated, the DNA-binding potential of this factor also appears to be regulated by phosphorylation [29]. The activator of the insulin E1 element is a heterodimer, composed of proteins in the basic helix–loop–helix family that are enriched in islets (NeuroD1 [30]/BETA2 [31]) and generally distributed (HEB [32] and E2A [33–35]).

Gene ablation experiments performed in mice on PDX-1 and NeuroD1 has clearly established a key role for each in pancreatic development. Thus, the expression of PDX-1 in precursor cells appears to be essential for the development of both the endocrine and exocrine compartments of the pancreas [36,37], whereas NeuroD1 [38] acts at a later stage of pancreatic development, involving islet cell formation and maintenance. Mutations in NeuroD1 [38,39] and PDX-1 [22,40,41] cause diabetes in both humans and mice, providing further support for each as a central regulator of β-cell physiological function.

In contrast to PDX-1, little is known about how glucose stimulates NeuroD1 activity. Here we show that NeuroD1 is in the nucleus under stimulating glucose conditions, and predominantly cytoplasmic under non-stimulating. Our results also suggest that the MEK–ERK signalling pathway mediates the increased transcriptional and nuclear localization activity of NeuroD1 in response to glucose. We propose phosphorylation influences NeuroD1 activation by affecting partitioning into the nucleus, and possibly through interactions with other transcriptional regulatory factors.

2. Materials and methods

2.1. MIN6 cell transfections

MIN6 cells [42] were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4500 mg glucose/l (Gibco) with 15% fetal calf serum (FCS; Gibco), penicillin (100 U/ml), streptomycin (100 mg/ml) (Gibco) and 50 μM β-mercaptoethanol. Cells between passages 26 and 31 were transfected in 6-well dishes using Lipofectamine 2000 (Promega) in OPTIMEM (Gibco) as described by the manufacturer. Briefly, cell at 70–80% confluency were transfected with 5.5 μg of total DNA (3 μg of G5b-CAT (chloramphenicol acetyl transferase) reporter, 1.5 μg Gal4-NeuroD156-355, 1 μg pGL2RSV luciferase (as a control for

*Corresponding author. Fax: (45)-44438000.
E-mail address: hvp@novo.dk (H.V. Petersen).

[1] Present address: University of Colorado, Barbara Davis Center for Childhood Diabetes, 4200 E 9th Avenue, 2M21D, P.O. Box B-140, Denver, CO 80262, USA.

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the transfection efficiency) together with 15 μl of Lipofectamine 2000. (The Gal4-NeuroD1 construct contains amino acids 156–356 of NeuroD1 fused in-frame to the to the DNA-binding domain of the *Saccharomyces cerevisiae* GAL4 transcription factor [43]. Four hours later the transfection mix was removed by addition of 2 ml of RPMI 1640 (Gibco; 0 mM glucose)+10% FCS. Glucose at either 3 or 20 mM was then added. The cells were harvested after 16 h and luciferase (Promega) and CAT assays performed [44]. The CAT activity of each sample was normalized to the protein content (Bio-Rad) of the extract.

2.2. Sub-cellular localization of NeuroD

MIN6 cells (passage 26–31) at 70–80% confluency were transfected in 200 ml culture flasks using either 40 μg hemagglutinin (HA)-NeuroD1 or green fluorescence protein (GFP)-NeuroD1 and 120 μl Lipofectamine 2000. The media was changed after 4 h to DMEM containing 15% FCS, 4500 mg/l glucose and 50 μM β-mercaptoethanol and incubated for an additional 16–20 h. The cells were then trypsinized and seeded on chamber slides. After an overnight incubation, the cells were washed in RPMI 1640 (0 mM glucose)+10% FCS, and media added containing either 3 or 20 mM glucose. The cells were fixed in 1% PFA after an overnight incubation. Immunocytochemistry was performed as described [45] using a monoclonal antibody to HA from Babco (0.5 μg/ml) and a FITC-conjugated secondary antibody 1:100. A Hamamatsu C5810 cooled CCD camera on a BX51 Olympus microscope was used to record images. Images were captured with Image Pro software and processed in Adobe Photoshop 5.0.

2.3. DNA constructs

2.3.1. pBK-RSV-NeuroD1. The rat NeuroD1 cDNA was cloned into pBK-RSV (Stratagene) using oligo-dT primed cDNA obtained by RT-PCR from the MSL-G2-IN rat insulinoma [46] and specific 5′ (5′-CCTGTTGGATCCAGGAAGTGGAAACATGA-3′) and 3′ (5′-TGAAACTCGAGAGCCTCTAATCGTGAAAGAT-3′) primers. The resulting PCR fragment was *Bam*HI and *Xho*I digested and ligated into the *Bam*HI and *Xho*I site of pBK-RSV.

2.3.2. HA-NeuroD1. pBK-RSV-NeuroD1 was digested with *Xba*I and *Kpn*I and a 700 bp NeuroD fragment purified. A PCR fragment containing the additional 380 bp of NeuroD and a new *Bam*HI and *Kpn*I site was obtained using the 5′ primer: 5′-ATGGGAGGATCCACCAAATCATAC3-′ and the 3′ primer: 5′-GAGTAGCAGGGTACCACCTTTCTC3-′. The PCR fragment was digested with *Bam*HI and *Kpn*I and purified. The 700 bp DNA fragment and the PCR fragment were ligated together into the *Bam*HI and *Xba*I site of the pcDNAI-Amp-HA vector (which was obtained by excising Ngn3 from pcDNAI/Amp-HA-Ngn3 using *Bam*HI and *Xba*I (a kind gift from F. Guillemot)).

Point mutations within HA-NeuroD1 were introduced using the QuickChange site-directed mutagenesis kit (Stratagene) with the following oligonucleotides (the mutated nucleotides are in bold and italicized): HA-NeuroDS259A: F: 5′-CTTCTTTGAA***GC***CCCCCTAACT-3′; R: 5′-AGTTAGGGGG***GC***TTCAAAGAAG-3′, HA-NeuroDT262A: F: 5′-AAAGCCCCCTA***G***CTGATTGCACC-3′; R: 5′-GGTGCAATCAG***C***TAGGGGGCTTT-3′, HA-NeuroDS266A: F: 5′-TGATTGCACC***GC***CCCTTCCTTT-3′; R: 5′-AAAGGAAGGG***GC***GGTGCAATCA-3′, HA-NeuroDS274A: F: 5′-ACGGACCCCTC***GC***CCCGCCGCTCA-3′; R: 5′-TGAGCGGCGGG***GC***GAGGGGTCCGT-3′, HA-NeuroDS274D: F: 5′-ACGGACCCCT***GGAT***CCGCCGCTCA-3′; R: 5′-TGAGCGGCGG***ATCC***AGGGGTCCGT-3′.

2.3.3. GFP-NeuroD1. NeuroD1 sequences were cut out from HA-NeuroD using *Xba*I and *Bam*HI and cloned into the *Xba*I and *Bam*HI site in the pECFG-C1 vector (Clontech).

3. Results

3.1. Partitioning of NeuroD1 into the nucleus is influenced by glucose

To investigate if glucose levels affected the sub-cellular localization of NeuroD1, HA and GFP tagged versions of the factor were constructed and transiently transfected into MIN6 cells. Both tagged constructs were principally located in the cytoplasm at low, non-stimulating glucose concentrations (3 mM; Fig. 1A,B), and in the nucleus under stimulating conditions (20 mM; Fig. 1C,D). Quantification revealed that 40–45% of tagged NeuroD1 was in the nucleus of 3 mM glucose-treated cells, as compared to 80–90% in 20 mM (Fig. 2). Furthermore, transfection of primary isolated islet cells with HA-NeuroD1 showed only expression of the HA-NeuroD in the nucleus of islet cells grown in 20 mM glucose (data not shown).

To examine whether the sub-cellular redistribution of NeuroD1 was also dependent on the MEK–ERK, PI3K, or p38SAPK signalling, HA-NeuroD1-transfected MIN6 cells

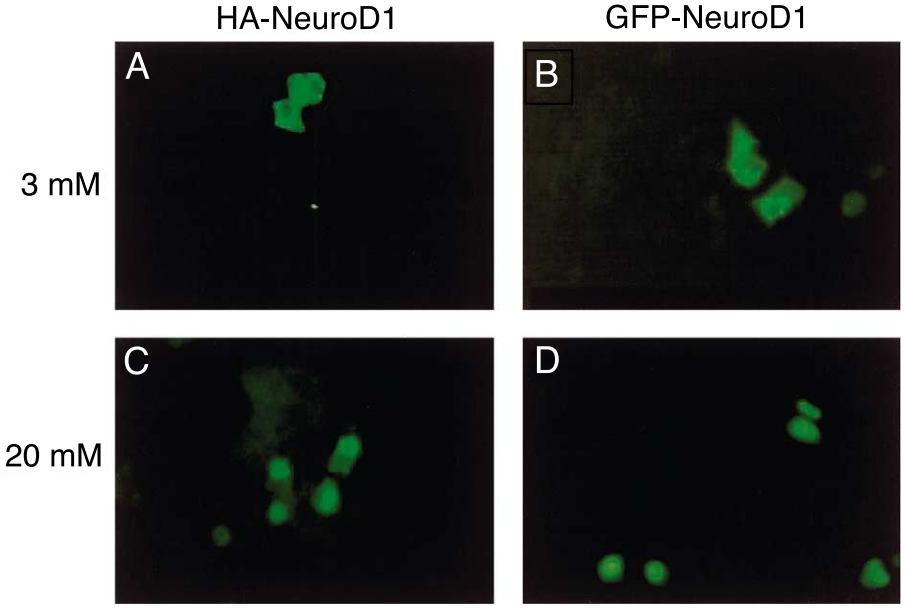

Fig. 1. Glucose induces the translocation of NeuroD1 into the nucleus. MIN6 cells were transiently transfected with (A,C) HA-NeuroD1 or (B,D) GFP-NeuroD1. The transfection cells were preincubated for 1 h in 0 mM glucose followed by 16 h in 3 or 20 mM glucose. The fixed HA-NeuroD1-transfected cells were stained for NeuroD1 expression using an antibody against HA (Babco) followed by a FITC-conjugated secondary antibody, whereas the GFP-Neuro1-transfected cells were examined directly by fluorescence microscopy using a FITC filter.

were treated with pathway-specific inhibitors (MEK, 50 μM PD98059; PI3K, 20 μM LY294002; p38SAPK, 20 μM SB203580). None of the inhibitors had any effect at 3 mM glucose whereas the MEK inhibitor PD98059 decreased the number of nuclear NeuroD1-expressing cells in 20 mM glucose from 90 to 65% (Fig. 2, $P < 0.03$), suggesting that activation of the MEK–ERK pathway stimulates nuclear localization of NeuroD1. In contrast, neither LY294002 nor SB203580 had a signification effect.

3.2. Serine 274 of NeuroD1 is involved in the nuclear translocation response to glucose

To begin to identify the amino acid(s) in NeuroD1 that mediates nuclear translocation in response to glucose, the potential ERK phosphorylation sites at serine 259, threonine 262, serine 266, and serine 274 were inactivated by mutating each to alanine. Their effect on sub-cellular distribution of NeuroD1 was determined immunohistochemically with anti-HA antibody in transfected cells grown in 25 mM glucose. The NeuroD1 mutants at serine 259, threonine 262, and serine 266 had a similar cytoplasmic staining fraction when compared to the wild type (Fig. 3). In contrast, the serine to alanine mutation at position 274 resulted in a marked increase in the number of cells harboring NeuroD1 in the cytoplasm. Importantly, a serine 274 to aspartic acid (S274D) mutation restored wild type-like activity (Fig. 3); this type of mutation is believed to mimic the phosphorylated state. Together these results demonstrate that NeuroD1 is translocated to the nucleus in response to stimulating glucose concentrations, a process that is partially dependent upon phosphorylation at serine 274 by the MEK–ERK signalling pathway.

3.3. NeuroD1 trans-AD activity is glucose-responsive and dependent upon the MEK–ERK signalling pathway

To determine if the transactivation potential of NeuroD1

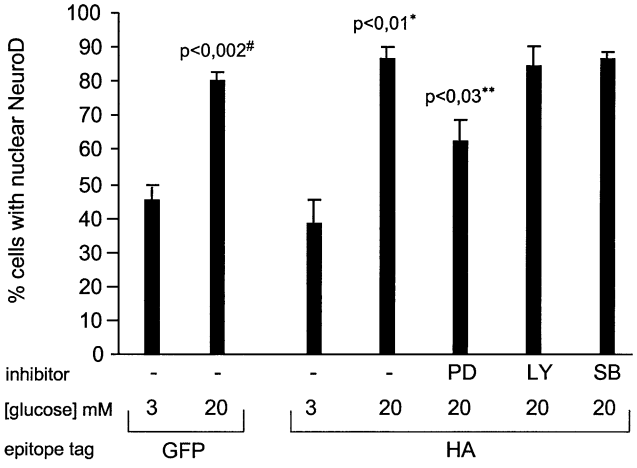

Fig. 2. The MEK-specific PD98059 inhibitor reduces the level of nuclear NeuroD1. Fraction of MIN6 cells containing nuclear GFP-NeuroD1 or HA-NeuroD1. The total number cytoplasmic and nuclear GFP-NeuroD1- or HA-NeuroD1-transfected cells were counted and percent of nuclear positive cells calculated as a fraction of the total. HA-NeuroD1-transfected cells were incubated in the presence and absence of 50 μM PD98059, 20 μM LY294002, and 20 μM SB203580. Results shown are the mean of three independent experiments. Error bars represent standard deviation. *Student's *t*-test on 3 vs. 20 mM glucose, **Student's *t*-test on 20 mM vs. 20 mM+PD.

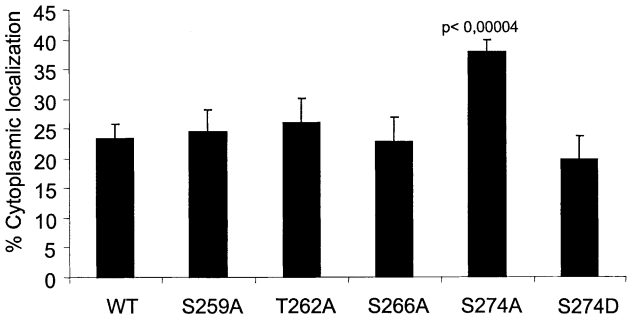

Fig. 3. Serine 274 in NeuroD1 mediates nuclear translocation. MIN6 cells were transiently transfected with the wild type and S259A, T262A, S266A, S274A, and S274D mutant HA-NeuroD1 and grown in the presence of 25 mM glucose. The HA-NeuroD1-transfected cells were stained and counted for NeuroD1 expression as described in Fig. 2. Results shown are the mean of five independent experiments. Error bars represent standard deviation. *Student's *t*-test on HA-NeuroDS274A compared to wild type (WT).

was regulated by glucose, the C-terminal AD-containing region (i.e. amino acids 156–355) was analyzed as a fusion with the DNA-binding domain of the *S. cerevisiae* Gal4 transcription factor. Gal4-NeuroD 156–355 was co-transfected into MIN6 cells together with a CAT reporter plasmid containing five GAL4 DNA-binding sites upstream of the E1b TATA box (G5b-CAT). Gal4-NeuroD 155–356 was roughly threefold more active in 20 mM glucose than 3 mM (Fig. 4, $P < 0.02$). This level of stimulation is similar to insulin enhancer-driven reporter constructs in MIN6 cells [24]. In contrast, the relative amount of Gal4-NeuroD nuclear protein was unaffected by these conditions (data not shown).

Specific MEK–ERK, PI3K, or p38SAPK signalling inhibitors were next tested to determine their involvement in Gal4-NeuroD 156–355 activation. Only the MEK-specific PD98059 inhibitor reduced glucose-inducible expression; inhibition was to approximately 50% of wild type (Fig. 4, $P < 0.05$). In contrast, NeuroD1 AD activity tended to increase in both 3 and 20 mM glucose, without significantly affecting the fold glucose activation in the presence of the PI3K inhibitor, LY294002. Collectively, these results suggested that stimulating glucose conditions influence both the level and activity of NeuroD1 in the nucleus. Furthermore, that the MEK–ERK pathway appears to mediate these processes.

4. Discussion

NeuroD1 appears to play a vital role in both β-cell-type-specific and glucose-regulated insulin gene transcription [47]. Mutations within this factor also contribute to β-cell dysfunction in type 2 diabetes [39]. Our objective here was to begin to define how NeuroD1 activity is controlled in glucose-stimulated β-cells. We show that the AD and nuclear partitioning potential of NeuroD1 are induced by glucose. In addition, the ability of PD98059 to reduce both of these activities suggests that MEK–ERK signalling is intimately involved. In contrast, NeuroD1 activity was unaffected by specific inhibitors of the PI3K and p38 SAP kinase pathways, emphasizing the particular significance of MEK–ERK signalling in regulation.

Two distinct tagged NeuroD1 fusion proteins, HA-NeuroD1 and GFP-NeuroD1, were constructed and used to investigate whether glucose affected sub-cellular localization. Importantly, their glucose response pattern was almost iden-

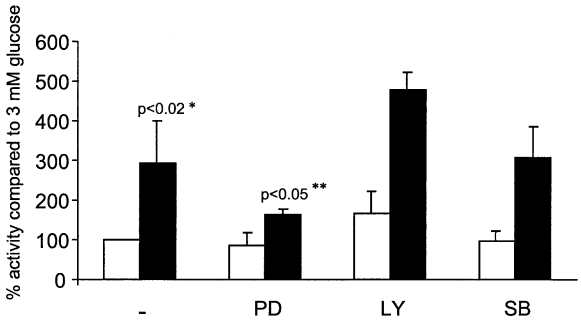

Fig. 4. The AD potential of NeuroD1 is stimulated by glucose. MIN6 cells co-transfected with Gal4-NeuroD (156–355) and G5bCAT were incubated with and without 50 μM PD98059, 20 μM LY294002, and 20 μM SB203580 in the presence of 3 mM (white bars) or 20 mM glucose (black bars). The results shown are the mean of at least three independent experiments. Error bars represents standard deviation. *Student's *t*-test between 3 mM and 20 mM glucose, **Student's *t*-test between 20 mM and 20 mM+PD.

tical. Thus, the number of MIN6 cells contain nuclear NeuroD1 increased by roughly two-fold in response to 3 versus 20 mM glucose (Fig. 2). In addition, this activity was significantly and specifically attenuated by treatment with the MEK inhibitor PD98059. The involvement of ERKs in β-cell function is also supported by two independent observations. First, glucose stimulates both ERK1 and ERK2 in MIN6 cells [48]. We have confirmed these findings and have also shown that neither PKB nor the stress-stimulated p38 SAP kinase was activated by glucose (data not shown). Second, PD98059 reduces insulin enhancer-mediated activation in response to glucose [49]. Collectively, these results strongly indicate that phosphorylation of NeuroD1 by ERK1 and/or ERK2 plays a key role in regulation.

Although we have not directly demonstrated that NeuroD1 is phosphorylated by the ERK1 or ERK2, the effect of mutating consensus phosphoacceptor sites within the protein would indicate that this is the case. Thus, nuclear partitioning was only affected by a dysfunctional mutation at the potential phosphoacceptor site at serine 274, and not by others within the C-terminal AD (i.e. serine 259, threonine 262, and serine 266) (Fig. 3). Significantly, replacing serine 274 with an aspartic acid residue did not affect nuclear transport. The negative charge of the aspartic acid likely mimicked phosphorylation of serine 274, strongly suggesting that this modification is directly involved in controlling the sub-cellular distribution of NeuroD1. The mechanisms regulating NeuroD1 partitioning between the cytoplasm and nuclei are unknown. Inspection of NeuroD1 indicates that the protein contains a nuclear localization (NLS) and nuclear export (NES) sequence, both of which are located outside the C-terminal AD region [50]. We are currently investigating how the NLS, NES and serine 274 influence the sub-cellular distribution of NeuroD1. Phosphorylation also regulates the sub-cellular localization of other transcription factors, including nuclear factor of activated T-cells [51–53], AFX, FKHR, and FKHR-L1 [54–60], and the STAT proteins [61].

The activity of the GAL4 chimera containing the C-terminal ADs of NeuroD1 was also responsive to both glucose and ERK signalling (Fig. 4). However, in contrast to sub-cellular localization, Gal4-NeuroD 156–355 activation was insensitive to the serine 274 mutations as well as those at serine 259, threonine 262, or serine 266 (data not shown). Presumably, this GAL4 NLS mediates constitutive transport of this chimera to the nucleus, since the potential NeuroD1 NLS was not present. As a consequence of the site-directed mutant analysis, it is unclear if the ERKs directly or indirectly influence the AD activity of NeuroD1. We are examining if interactions between the insulin gene coactivator, p300, and the C-terminal region of NeuroD1 may be involved in potentiation by glucose [28,29].

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References

[1] Sander, M. and German, M.S. (1997) J. Mol. Med. 75, 327–340.
[2] Stein, R. (1993) Trends Endocrinol. Metab. 4, 96–101.
[3] Sander, M., Neubuser, A., Kalamaras, J., Ee, H.C., Martin, G.R. and German, M.S. (1997) Genes Dev. 11, 1662–1673.
[4] Peshavaria, M., Gamer, L., Henderson, E., Teitelman, G., Wright, C. and Stein, R. (1994) Mol. Endocrinol. 8, 806–816.
[5] German, M.S., Wang, J., Chadwick, R.B. and Rutter, W.J. (1992) Genes Dev. 6, 2165–2176.
[6] Shieh, S.Y. and Tsai, M.J. (1991) J. Biol. Chem. 266, 16708–16714.
[7] Sharma, A. and Stein, R. (1994) Mol. Cell. Biol. 14, 871–879.
[8] Crowe, D. and Tsai, M. (1989) Mol. Cell. Biol. 9, 1784–1789.
[9] Karlsson, O., Edlund, T., Moss, J., Rutter, W. and Walker, M. (1987) Proc. Natl. Acad. Sci. USA 84, 8819–8823.
[10] Whelan, J., Cordle, S., Henderson, E., Weil, P. and Stein, R. (1990) Mol. Cell. Biol. 10, 1564–1572.
[11] Edlund, T., Walker, M.D., Barr, P.J. and Rutter, W.J. (1985) Science 230, 912–916.
[12] German, M.S. and Wang, J. (1994) Mol. Cell. Biol. 14, 4067–4075.
[13] Melloul, D., Ben-Neriah, Y. and Cerasi, E. (1993) Proc. Natl. Acad. Sci. USA 90, 3865–3869.
[14] Odagiri, H., Wang, J. and German, M.S. (1996) J. Biol. Chem. 271, 1909–1915.
[15] Sharma, A., Fusco-DeMane, D., Henderson, E., Efrat, S. and Stein, R. (1995) Mol. Endocrinol. 9, 1468–1476.
[16] Petersen, H.V., Serup, P., Leonard, J., Michelsen, B.K. and Madsen, O.D. (1994) Proc. Natl. Acad. Sci. USA 91, 10465–10469.
[17] Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S. and Montminy, M. (1993) Mol. Endocrinol. 7, 1275–1283.
[18] Miller, C.P., McGehee Jr., R.E. and Habener, J.F. (1994) EMBO J. 13, 1145–1156.
[19] Ohlsson, H., Karlsson, K. and Edlund, T. (1993) EMBO J. 12, 4251–4259.
[20] Serup, P., Jensen, J., Andersen, F.G., Jorgensen, M.C., Blume, N., Holst, J.J. and Madsen, O.D. (1996) Proc. Natl. Acad. Sci. USA 93, 9015–9020.
[21] Watada, H. et al. (1996) Diabetes 45, 1826–1831.
[22] Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K. and Edlund, H. (1998) Genes Dev. 12, 1763–1768.
[23] MacFarlane, W.M., Read, M.L., Gilligan, M., Bujalska, I. and Docherty, K. (1994) Biochem. J. 303, 625–631.
[24] Petersen, H.V., Peshavaria, M., Pedersen, A.A., Philippe, J., Stein, R., Madsen, O.D. and Serup, P. (1998) FEBS Lett. 431, 362–366.
[25] Shushan, E.B., Cerasi, E. and Melloul, D. (1999) DNA Cell. Biol. 18, 471–479.
[26] Rafiq, I., Kennedy, H.J. and Rutter, G.A. (1998) J. Biol. Chem. 273, 23241–23247.
[27] Rafiq, I., da Silva Xavier, G., Hooper, S. and Rutter, G.A. (2000) J. Biol. Chem. 275, 15977–15984.
[28] Macfarlane, W.M., McKinnon, C.M., Felton-Edkins, Z.A., Cragg, H., James, R.F. and Docherty, K. (1999) J. Biol. Chem. 274, 1011–1016.

[29] Matsuoka, T., Zhao, L. and Stein, R. (2001) J. Biol. Chem. 276, 22071–22076.
[30] Lee, J.E., Hollenberg, S.M., Snider, L., Turner, D.L., Lipnick, N. and Weintraub, H. (1995) Science 268, 836–844.
[31] Naya, F., Stellrecht, C. and Tsai, M. (1995) Genes Dev. 9, 1009–1019.
[32] Peyton, M., Moss, L. and Tsai, M. (1994) J. Biol. Chem. 269, 25936–25941.
[33] Aronheim, A., Ohlsson, H., Park, C.W., Edlund, T. and Walker, M.D. (1991) Nucleic Acids Res. 19, 3893–3899.
[34] Cordle, S., Henderson, E., Masuoka, H., Weil, P. and Stein, R. (1991) Mol. Cell. Biol. 11, 1734–1738.
[35] German, M., Blanar, M., Nelson, C., Moss, L. and Rutter, W. (1991) Mol. Endocrinol. 5, 292–299.
[36] Ahlgren, U., Jonsson, J. and Edlund, H. (1996) Development 122, 1409–1416.
[37] Offield, M.F., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Magnuson, M.A., Hogan, B.L. and Wright, C.V. (1996) Development 122, 983–995.
[38] Naya, F.J., Huang, H.P., Qiu, Y., Mutoh, H., DeMayo, F.J., Leiter, A.B. and Tsai, M.J. (1997) Genes Dev. 11, 2323–2334.
[39] Malecki, M.T. et al. (1999) Nat. Genet. 23, 323–328.
[40] Dutta, S., Bonner-Weir, S., Montminy, M. and Wright, C. (1998) Nature 392, 560.
[41] Stoffers, D.A., Ferrer, J., Clarke, W.L. and Habener, J.F. (1997) Nat. Genet. 17, 138–139.
[42] Miyazaki, J., Araki, K., Yamato, E., Ikegami, H., Asano, T., Shibasaki, Y., Oka, Y. and Yamamura, K. (1990) Endocrinology 127, 126–132.
[43] Peshavaria, M., Henderson, E., Sharma, A., Wright, C. and Stein, R. (1997) Mol. Cell. Biol. 17, 3987–3996.
[44] Gorman, C. (1985) in: DNA Cloning, A Practical Approach (Glover, D., Ed.), Vol. II, pp. 143–190, IRL Press, Oxford University, New York.
[45] Madsen, O., Nielsen, J., Michelsen, B., Westermark, P., Betsholtz, C., Nishi, M. and Steiner, D. (1991) Mol. Endocrinol. 5, 143–148.
[46] Madsen, O.D., Andersen, L.C., Michelsen, B., Owerbach, D., Larsson, L.I., Lernmark, A. and Steiner, D.F. (1988) Proc. Natl. Acad. Sci. USA 85, 6652–6656.
[47] Ohneda, K., Ee, H. and German, M. (2000) Semin. Cell. Dev. Biol. 11, 227–233.
[48] Benes, C., Roisin, M.P., Van Tan, H., Creuzet, C., Miyazaki, J. and Fagard, R. (1998) J. Biol. Chem. 273, 15507–15513.
[49] Benes, C., Poitout, V., Marie, J.C., Martin-Perez, J., Roisin, M.P. and Fagard, R. (1999) Biochem. J. 340, 219–225.
[50] Ullman, K.S., Powers, M.A. and Forbes, D.J. (1997) Cell 90, 967–970.
[51] Neilson, J., Stankunas, K. and Crabtree, G.R. (2001) Curr. Opin. Immunol. 13, 346–350.
[52] Graef, I.A., Mermelstein, P.G., Stankunas, K., Neilson, J.R., Deisseroth, K., Tsien, R.W. and Crabtree, G.R. (1999) Nature 401, 703–708.
[53] Beals, C.R., Sheridan, C.M., Turck, C.W., Gardner, P. and Crabtree, G.R. (1997) Science 275, 1930–1934.
[54] Takaishi, H. et al. (1999) Proc. Natl. Acad. Sci. USA 96, 11836–11841.
[55] Biggs III, W.H., Meisenhelder, J., Hunter, T., Cavenee, W.K. and Arden, K.C. (1999) Proc. Natl. Acad. Sci. USA 96, 7421–7426.
[56] Brownawell, A.M., Kops, G.J., Macara, I.G. and Burgering, B.M. (2001) Mol. Cell. Biol. 21, 3534–3546.
[57] Brunet, A. et al. (1999) Cell 96, 857–868.
[58] Brunet, A., Park, J., Tran, H., Hu, L.S., Hemmings, B.A. and Greenberg, M.E. (2001) Mol. Cell. Biol. 21, 952–965.
[59] del Peso, L., Gonzalez, V.M., Hernandez, R., Barr, F.G. and Nunez, G. (1999) Oncogene 18, 7328–7333.
[60] Kops, G.J., de Ruiter, N.D., De Vries-Smits, A.M., Powell, D.R., Bos, J.L. and Burgering, B.M. (1999) Nature 398, 630–634.
[61] Bromberg, J. and Darnell Jr., J.E. (2000) Oncogene 19, 2468–2473.